

Claims

1. Method for cloning a gene comprising the steps of:
- (i) Providing a replication-deficient baculovirus vector;
 - (ii) Providing a rescue vector encoding:
 - (a) a nucleic acid sequence which is capable of restoring replication in the replication-deficient baculovirus vector; and
 - (b) at least one gene to be cloned.
 - (iii) Causing the replication-deficient baculovirus vector and rescue vector to recombine to produce a replication-enabled baculovirus vector comprising the at least one gene to be cloned; and
 - (iv) Growing the replication-enabled baculovirus vector within a suitable invertebrate cell.
2. Method according to claim 1, wherein the invertebrate cell is an insect cell.
3. Method according to claim 1 or claim 2, wherein the replication-deficient baculovirus vector lacks a functional gene necessary for viral replication and the rescue vector comprises a gene necessary for restoring the functional gene.
4. Method according to claim 3, wherein the functional gene is selected from *lef-1*, *lef-2*, *lef-3*, *lef-4*, *lef-5*, *lef-6*, *lef-7*, *lef-8*, *lef-9*, *lef-10*, *lef-11*, *lef-12*, *dnapol*, *p143*, *p35*, *ie-1*, *ie-2*, *p47*, *ORF1629* and *pp31*, or a functional fragment or mutation thereof.
5. Method according to claim 4, wherein the functional gene is *lef-2* or a functional fragment or mutation thereof.
6. A method according to any preceding claim wherein the replication deficient baculovirus vector is capable of being maintained in an intermediate host.
7. A method according to claim 6, wherein the intermediate host is a yeast cell or a bacterial cell.

8. A method according to claim 6 or claim 7, wherein the replication deficient baculovirus vector comprises one or more nucleic acid sequences which enable the vector to replicate within the intermediate host.
9. A method according to any preceding claim wherein the recombination step (iii) takes place within the invertebrate cell.
10. A method according to any preceding claim, additionally comprising the step of (v) growing invertebrate cell so that the or each foreign gene is expressed within the cell.
11. A replication deficient baculovirus vector for use in a method according to any preceding claim.
12. A vector according to claim 11, additionally comprising one or more nucleic acid sequences which enable the vector to replicate within an intermediate host.
13. A vector according to claim 12, wherein the vector comprises one or more nucleic acid sequences selected from ARS-1, CEN-1 and a bacterial origin of replication (*ori*).
14. A vector according to any one of claims 11 to 13, in which at least a part of the naturally occurring polyhedrin gene has been deleted.
15. A vector according to any one of claims 11 to 14, wherein the vector is based upon AcMNPV.
16. A rescue vector for use in a method according to any one of claims 1 to 10 comprising (a) a nucleic acid sequence which is capable of restoring replication in a replication-deficient baculovirus, and (b) at least one site for insertion of a gene to be cloned.
17. A rescue vector according to claim 16, wherein the site for insertion of the gene to be cloned is operably linked to regulatory elements necessary for expression of said gene.

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18. A rescue vector according to claim 17, wherein the site for insertion of the foreign gene is operably linked to a promoter selected from a baculovirus polyhedrin promoter or a baculovirus p10 promoter.
19. An intermediate host cell comprising a replication-deficient baculovirus vector according to any one of claims 11 to 15.
20. A cell comprising a rescue vector according to any one of claims 16 to 18.
21. An invertebrate cell obtainable by a method according to any one of claims 1 to 10.
22. A kit for use in a method according to any one of claims 1 to 10 comprising a replication deficient vector according to any one of claims 11 to 15.
23. A kit according to claim 22 additionally comprising a rescue vector according to any one of claims 16 to 18.
24. ^a A kit for use in a method according to any one of claims 1 to 10 comprising a rescue vector according to any one of claims 16 to 18.
25. A kit according to any one of claims 22 to 24 additionally comprising one or more buffers, preservatives or stabilising agents.
26. An isolated nucleic acid molecule consisting of a sequence selected from:
AAATTCAGATATAAAGACGCTGAAAATCATTG,
TGATTTTCAGCGTCTTTATATCTGAATTT,
ATTTTCGCTCTAACATAGCACCCTAGGGATGTAC,
GTACATCCCTAGGGTGGTATGTTAGAGCGAAAATCAAA,
CTAGGGATTATAAATTTAATGAATTATTAATAAC,
GTATTTTAATAATTCATTAAATTTATAATCC.

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